VIRUS DISEASE OF SOCKEYE SALMON

Interim Report

SPECIAL SCIENTIFIC REPORT—FISHERIES No. 138

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Explanatory Note

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and Robert R. Rucker,
Fishery Biologists

Special Scientific Report: Fisheries No. 138

Washington, D. C.

December, 1954

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Since 1951 a disease, usually occurring in late spring or early summer, has caused severe losses in 3- to 12-month-old fingerling sockeye salmon in hatcheries in the State of Washington. The disease is characterized by an explosive outbreak, mortality usually 80 percent or greater, and a residual spinal deformity in a small percentage of the surviving fish, and its specificity for the one species of salmon, Oncorhynchus nerka. (The anadromous strain of this species is commonly known as sockeye, blueback, or red salmon, while the fresh-water strain is called kokanee or silver trout.) The etiological agent is believed to be a virus.

The potential seriousness of the disease was recognized in 1951 (Rucker, et al., 1953) when it occurred at two salmon hatcheries in the State of Washington. The following year the serious nature of this disease became even more apparent (Watson, 1954) when nearly two million sockeye fingerlings were lost during the epizootics which occurred at three of the hatcheries. In 1953, epizootics occurred at all five hatcheries which reared sockeye salmon in the State of Washington.

These epizootics have been of biological and economic significance. Because the building of the Grand Coulee Dam curtailed access to the natural spawning areas in the upper tributaries of the Columbia River, in 1940 the United States Fish and Wildlife Service established a program to relocate the spawning areas and to increase the runs by artificial propagation. By 1947 there had been a gradual increase in the number of adult sockeye salmon which returned to spawn in the tributaries of the Columbia River apparently as a result of hatchery operations. With the increase of adults it was possible to take a greater number of eggs for hatchery operations, and 2 to 6 million hatchery-reared sockeye salmon were released annually into the Columbia River system between 1947 and 1951. In 1952-53, however, 60 to 90 percent of the hatchery-reared fingerlings in the entire State died from this disease before being released. If this disease continues to cause severe losses in hatchery-reared fish, it may bring a serious economic loss to both the Federal Government and the fisheries industry.

Of academic interest is the fact that we are dealing with a virus that affects an aquatic vertebrate. Only a few such viruses are known or described at the present time. The virus diseases of fish are possibly the best documented of the virus diseases of aquatic vertebrates, but even so, there are comparatively few published reports of such diseases. Since virus diseases of fish have been reviewed recently (Watson, 1954), no references will be made to these reports since they seem to have no direct bearing on the present problem.

The objectives of this investigation were to describe the epizootiology and etiology, to determine the efficacy of chemotherapy and the effect of physical factors on the infectious agent. Despite the fact that there were and still are many points left undetermined and the evidence gathered was not always conclusive, sufficient information of interest was obtained to warrant the presentation of this interim report. A comprehensive study of this virus disease is currently in progress.

This report deals only with the epizootics which occurred at the five salmon hatcheries in the State of Washington, which are situated in widely separated parts of the state (see fig. 1):

Cook	)	
Entiat	)	
Leavenworth	)	U. S. Fish and Wildlife Service Hatchery
Winthrop	)	
Issaquah		Washington State Department of Fisheries Hatchery.

Each year, the normal procedure has been to trap and spawn fish in local streams in late September and early October, to hatch the eggs in hatchery troughs, and to rear the fry and fingerlings in troughs until the following April or sometimes as late as July. At that time, the majority of the fingerlings have been moved to outside rearing ponds, remaining there until the fall, when they have been planted in a lake having an outlet to the ocean. At the Winthrop and Cook hatcheries, however, the majority of the sockeye salmon were held through their second winter in rearing ponds or raceways before they were released into tributaries of the Columbia River.

The eggs were spawned from adults in September and hatched in December or January, but the fry did not begin to feed for an additional 30 days. The fingerlings were normally fed a diet consisting of beef, hog, and fish products.

As it was often necessary to supplement an established salmon run or even to establish a new run, eggs were often transferred from one hatchery to another in a different area, reared there, and subsequently released into lakes and streams in that area.

Fig. 1.--Map of the State of Washington showing the geographic location of hatcheries where epizootics occurred.

EPIZOOTIOLOGY

Record of Epizootics

In 1951, epizootics occurred in the sockeye-salmon populations at both the Leavenworth and Winthrop hatcheries, and in the kokanee-trout population at the Leavenworth hatchery (table 1). At Leavenworth the disease in the sockeye populations was restricted to 6 of 102 troughs, but at the Winthrop hatchery it was present in all sockeye troughs; at Leavenworth the disease was manifest also in 15 of the 37 kokanee troughs. Almost 100 percent of the infected sockeye populations died, but only 39.6 percent of the infected kokanees.

The seriousness of the disease became increasingly apparent in 1952 when three of the five hatcheries (Leavenworth, Winthrop, Cook) reported epizootics. The entire sockeye population became infected at each of these hatcheries, and the mortality at both Leavenworth and Cook exceeded 90 percent. Altogether, the number of sockeye fingerlings lost at these three stations during 1952 totaled 2,368,000, which represents a loss of 91.5 percent of all the sockeye fingerlings reared at these hatcheries between June and September, and more than 85 percent of all sockeye fingerlings reared in the State of Washington.

The epizootics were considerably more widespread in 1953, when the disease occurred at all five of the hatcheries that raise sockeye salmon in the State of Washington (see table 1). A total of 2,053,000 fish died during the epizootics, representing a loss of 65 percent of all the sockeye fingerlings reared in the State that year; the losses probably would have been more serious had not 441,000 fingerlings been released before the onset of the disease.

Mortality Pattern During an Epizootic

Under normal conditions, the average daily mortality of sockeye salmon fingerlings reared in a hatchery is about 0.01 percent of the total population. During epizootics the daily mortalities increased rapidly and in some populations reached 32 percent within 20 days after the initial increase in mortality. The cumulative mortality pattern was similar, in the majority of the epizootics, to that described by Watson (1954) for infected ponds at the Leavenworth hatchery in 1952. At this hatchery the daily mortality rose to 1 percent a day within 6 to 9 days after the onset of the disease. The cumulative mortality reached 50 percent within 16 to 22 days after the disease was first seen. Within 25 to 40 days after the onset of the disease, 90 percent of the fish were dead.

Table 1.--Summary of epizootics

Year	Station	Date of Onset	Total ponds	Total ponds infected	Total troughs	Total troughs infected	Total fish	fish infected	% Mort. Infected Fish	% Mort. Tot. No. of Fish	Average Water Temp. °F.
1951	Leaven.	Mar. 21	0	0	102	6	1,400,000	80,000	99.0	5.7	46
1951	Winthrop	Apr. 23	0	0	6	6	69,000	69,000	99.0	99.0	52
1951	* Leaven.	May 18	3	3	37	5	788,000	788,000	39.6	39.6	45
1952	Leaven.	June 3	11	11	0	0	2,200,000	2,200,000	94.0	94.0	48
1952	Winthrop	July 7	1	1	0	0	113,000	113,000	45.0	45.0	53
1952	Cook	Aug. 28	2 **	2 **	0	0	282,000	282,000	95.0	95.0	47
1953	Issaquah	Apr. 7	0	0	2	2	62,000	62,000	100	99.0	47
1953	Cook	Apr. 28	0	0	22	6	404,000	87,000	90.0	17.6	50
1953	Entiat	May 23	0	0	27	14	40,000	14,000	90.0	31.6	46
1953	Winthrop	June 20	1	1	11	0	109,000	90,000	59.4	45.5	50
1953	Leaven.	June 15	8	8	132	69	2,252,600	2,024,000	91.0	83.0	46

* Kokanee population

** Raceways

The exceptions to the typical mortality pattern occurred at the Winthrop hatchery in 1952 and 1953 (fig. 2), in the infected kokanee population at the Leavenworth hatchery in 1951, and in some of the troughs of sockeye fingerlings which became infected later in the summer at the Leavenworth hatchery in 1953.

The disease in the kokanee population at the Leavenworth station was first recognized in one trough on May 18, 1951 (Rucker, et al., 1953). The losses increased rapidly, and the fish in this trough were destroyed. On May 27, five troughs became infected, but the mortality rate did not rise rapidly. The fish in these troughs, as well as the fish in 37 troughs which showed no symptoms of the disease, were moved to three outside holding ponds on July 18. The mortality decreased, and by July 16 only 36 percent of the kokanees had died. Just before disposal of these fish on October 9, there was a significant rise in the mortality attributed to the disease, and the total mortality at time of disposal was 39.6 percent.

The virus disease at the Winthrop hatchery caused a 45-percent mortality in 1952 and a 59.4-percent mortality in 1953, in infected populations. When the disease became apparent at this hatchery in 1952, all the sockeye-salmon fingerlings were confined to one pond. Shortly after the onset of the disease, half of the fish were transferred to a clean rearing pond and the other half to a canal. The volume of water flowing in the canal allowed for a much greater flushing action than found in the rearing pond. About 45 percent of the fish placed in the canal died within 30 days; after that, there were no significant losses. In contrast, the mortality of the fish in the clean rearing pond continued at a low level for the next 90 days until the total mortality reached 45 percent; the death rate then returned to normal.

In 1953, the 90,000 fingerlings in the Winthrop hatchery, reared in one holding pond (pond No. 120), showed the first evidence of the disease on June 20. After the disease was recognized, the fish were not moved either to the clean rearing ponds or the canal as had been done the previous year; instead, they were retained in the original pond. Sixty-five days after the recognition of the disease in the pond, 59.4 percent of the fish had died. (fig. 2).

The majority of the 69 troughs and 8 ponds which became infected at the Leavenworth hatchery in 1953 showed the initial symptoms of the disease between June 15 and July 15. Late in August, however, infection appeared in healthy fish adjacent to a trough in which there were diseased fish, and in October 3,000 healthy fingerlings in another trough were experimentally infected to determine the virulence of the disease during this period. The fish in the troughs in which the initial onset of the disease occurred between June 15 and June 30 suffered a mortality of more than 95 percent. The total mortality in the troughs infected between

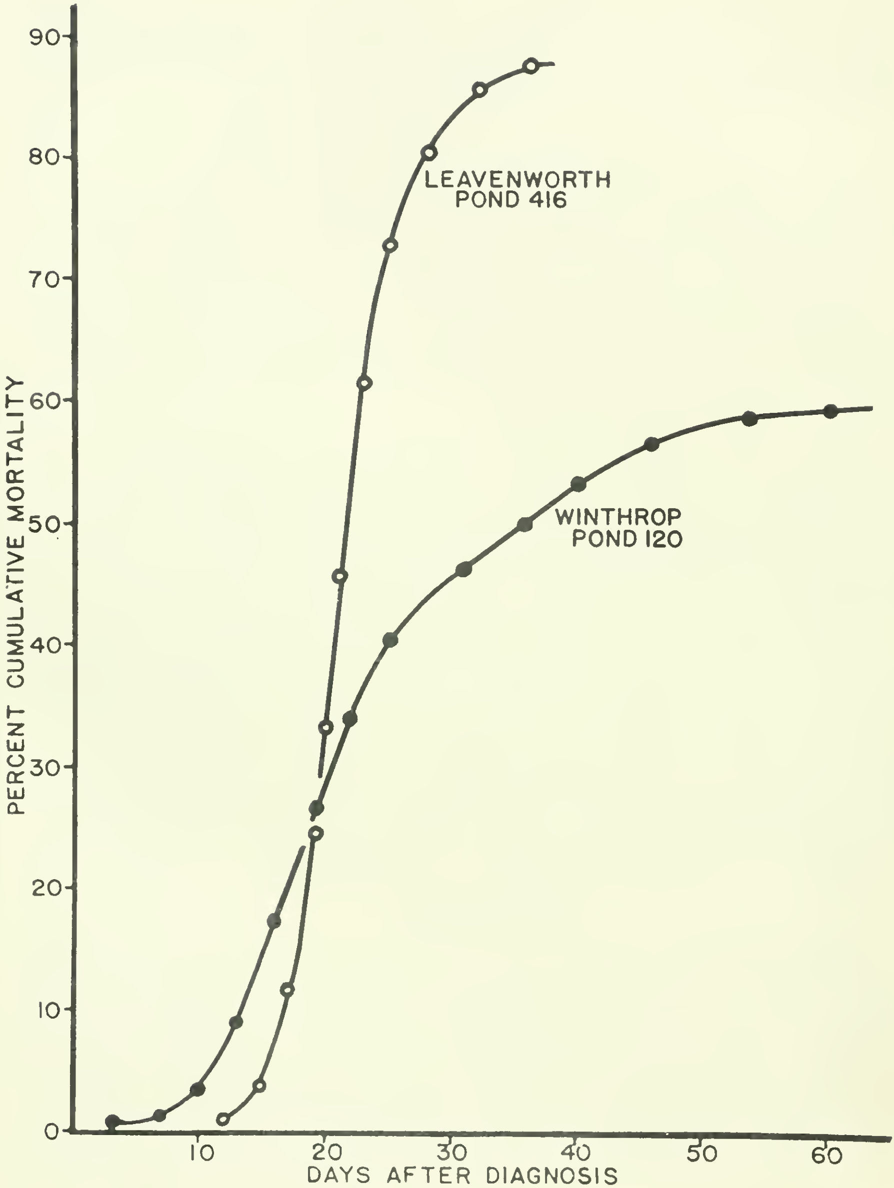

Fig. 2.--Comparison of typical (Leavenworth 1953) and atypical (Winthrop 1953) pond mortality rates during an epizootic.

July 1 and July 15 was 90 percent, and the total mortality in the troughs in which fish became infected between July 15 and July 30 was 70 to 80 percent. The trough in which fish became infected in August had a 37-percent mortality, while the trough in which fish became infected in October had only a 22-percent mortality (fig. 3).

Distribution of Infected Fish in Hatcheries

The incidence of epizootics was greater in ponds than in troughs (see table 1). When an epizootic occurred in a hatchery where fingerlings were being reared in ponds and troughs, the infection was manifest in all ponds, but usually not in all troughs. The only hatchery where fish were reared in several ponds was at the Leavenworth hatchery in 1952 and 1953, where all ponds became infected. No sequence was observed in the order in which they became infected to indicate that the virus was being disseminated from diseased to healthy ponds. However, the virus could have been spread easily between ponds by scavenging birds which were observed dropping moribund fish.

Rarely did all the fish in all of the troughs become infected. Rucker, et al., (1953) reported that when the disease was first observed at Leavenworth in 1951 only the fish in six troughs became infected, although sockeye fingerlings were being reared in 102 troughs. In 1952, when the epizootic occurred all of the fingerlings were being reared in rearing-ponds, and the fish in all ponds became infected. However, at Leavenworth in 1953 a large number of fingerlings were being reared in 132 hatchery troughs and 8 ponds when the epizootic occured in fish in 69 of the troughs and all of the ponds. In the same year at the Cook hatchery, the six troughs which became infected were in the center of the hatchery. At this hatchery it appeared that the virus was being disseminated from diseased to healthy troughs. At the Entiat hatchery in 1953, the majority of the diseased troughs were at one end of the hatchery (see fig. 4), while at the Leavenworth hatchery, in that same year, most of the infected troughs were in two of the six sections of the hatchery where sockeye fingerlings were being reared (see fig. 5). The order in which the troughs became infected at these two hatcheries did not indicate that the disease was spread from diseased to healthy troughs.

Symptomatology

During an epizootic most of the fish appeared normal until just before death. Two to ten hours before they died the fish became sluggish and were easily caught -- some of the fish became dark in color. Approximately 10 percent of the fish became side-swimmers, apparently the result of a partial paralysis. The left-handed swimmers swam in circles to the left and the right-handed in circles to the right. The fish in this condition became very excitable on occasion and dashed around wildly in an erratic manner, often jumping out of the water. A spasm of this nature usually lasted 45 seconds; then, as if exhausted from the sudden burst of activity, the fish

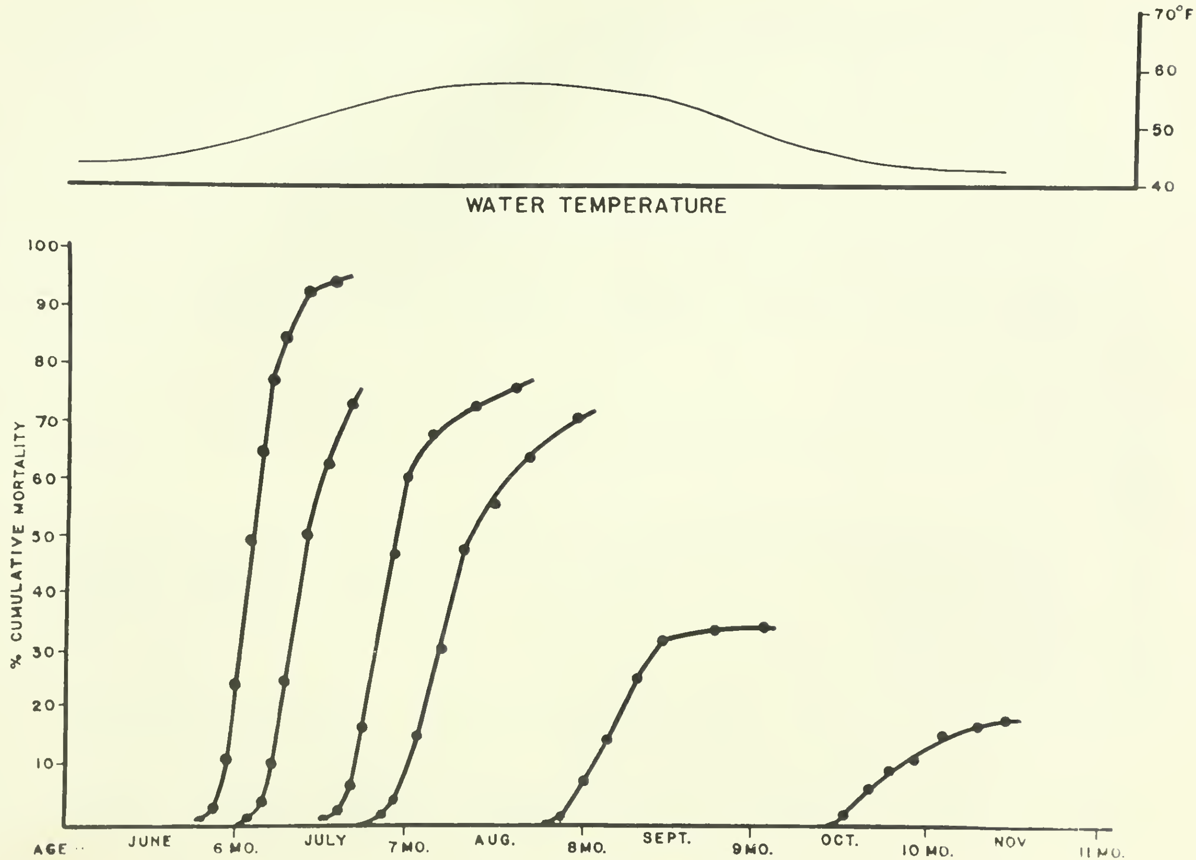

Fig. 3.--Representative trough mortalities at the Leavenworth hatchery in 1953. Curve represents mortalities in individual troughs becoming infected with the virus disease at different times throughout the season.

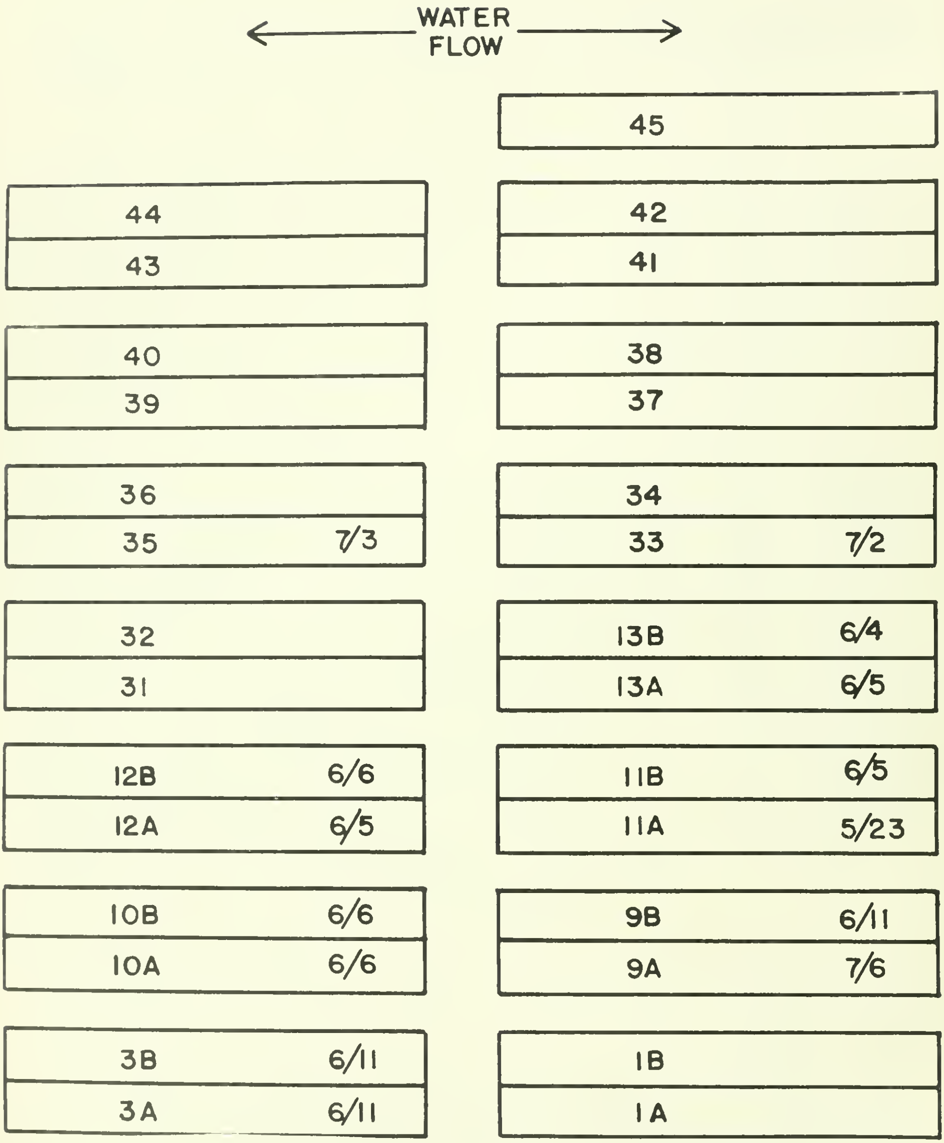

Fig. 4.--Distribution of infected troughs, Entiat hatchery, 1953, and dates of diagnosis.

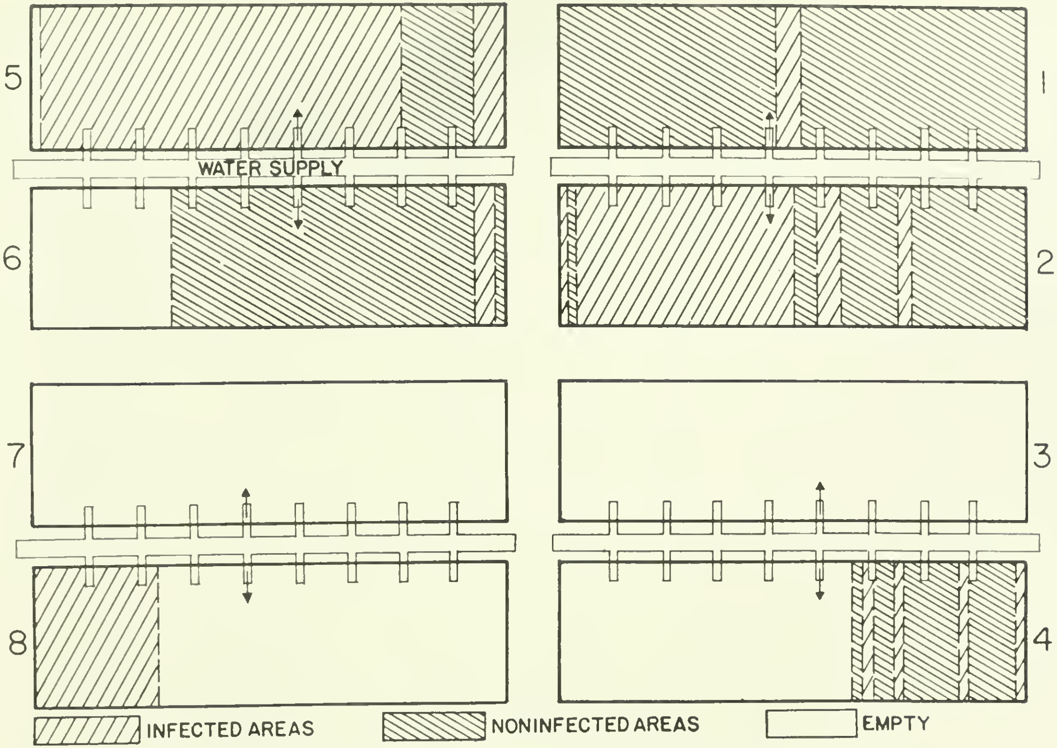

Fig. 5.--Distribution of infected troughs, Leavenworth hatchery, 1953

Section	Number troughs infected per section
1	3
2	21
3	0
4	5
5	26
6	2
7	0
8	12

sank to the bottom and remained motionless until the next spasm. The side-swimmers were hypersensitive and were more difficult to catch than healthy fish. When one of the fish afflicted in this manner was held, the skin of the fish exhibited a peculiar quivering reaction.

Fish which died at the onset of the disease showed no symptoms other than those just described. Shortly after the disease was recognized, however, hemorrhagic and eroded areas were noticed at the base of the pelvic and pectoral fins and occasionally at the base of the dorsal fins of moribund fish. The incidence of external hemorrhagic areas was greatest during the height of the disease. Later, when the epizootic began to subside, there was a marked decrease in the relative number of fish dying with hemorrhagic areas.

In an epizootic, spinal deformities (resembling scoliosis and lordosis in man) developed after 85 percent of the population was dead. Most of these fish died eventually, but some became side-swimmers before they died. Some of the fish with spinal deformities were kept alive for a period of 3 months. Their deformities often became more accentuated during this period.

Factors Possibly Correlated With the Onset of the Disease

Source of the Virus

The foremost problem concerning these epizootics is the original introduction of the virus into a hatchery population. The success of the control measures may depend entirely upon the ability to determine the initial source of the virus. Possible sources through which the infectious agent could be introduced are food, airborne and aquatic vectors, and the eggs from which the fish develop.

Food.--As previously stated, the diet fed to the sockeye salmon fingerlings in hatcheries consisted of a ground, homogenized mixture of beef, hog, and fish products. Because of the usual species specificity of viruses, the fish products portion of the diet is considered a more likely introductory source than either the hog or beef product portions. The fish products usually fed were either salmon carcasses, viscera, or eggs, or a combination of these products.

The viscera and eggs were from salmon caught in waters in the vicinity of Alaska, British Columbia, or Washington. The fish were taken to canneries where the viscera and eggs were removed. These products were shipped to fish product distributors where they were frozen in 50-pound blocks. The viscera and egg blocks were shipped to hatcheries where they were stored at temperatures below freezing until ready for use.

Each hatchery had its own cold-storage facilities for the storage of the various constituents of the diets. However, since Winthrop and Entiat hatcheries had limited cold-storage facilities, the majority of their viscera was stored at the Leavenworth hatchery. Periodically, limited amounts of the viscera were shipped to the Entiat and Winthrop hatcheries. Except in special instances, no effort was made to segregate the fish products used at any of these three hatcheries. Lots of viscera at Leavenworth were treated as a common supply for the Entiat, Winthrop, and Leavenworth hatcheries. Blocks from any one lot were used at each of the three hatcheries.

Little information is available concerning the species of salmon from which the viscera was obtained. It is believed that the majority of the viscera fed at the Winthrop, Entiat, and Leavenworth hatcheries in 1953 was from pink and sockeye salmon, and the eggs fed at the Cook hatchery from chinook and chum salmon.

The diet fed at the Leavenworth and Winthrop hatcheries consisted of approximately 30 percent salmon viscera and 70 percent a mixture of one or more of the following products: beef-liver, hog-liver, hog-spleen, or beef lungs. The diet fed at the Cook hatchery did not include any viscera, but rather a mixture of 30 percent salmon eggs and 70 percent beef and hog products. The diet at the Issaquah hatchery included fish viscera, fish carcasses, and beef-liver. Although all of the troughs of fish were fed the same diet at each hatchery, not all of the troughs became infected (table 1).

There were two groups of sockeye fingerlings reared at the Entiat hatchery in 1953. In the first group, 12 of the 14 troughs were fed a diet of beef-liver, salmon viscera, and additional fish products of hake and arrowtoothed halibut in various proportions. The diet for the fish in the two remaining troughs of this group consisted of pure beef-liver -- no fish products were incorporated. The viscera for the first group of fingerlings were from a lot set aside early in the season in order that a minimum variation be present in the viscera for the planned nutritional experiments. All troughs in the first group of fish (fig. 4, troughs 1A through 13B) became infected with the exception of the two troughs which were fed a pure beef-liver diet (fig. 4, troughs 1a and 1b).

The second group of sockeye fingerlings at the Entiat hatchery (fig. 4, troughs 31-45) received a diet similar to that fed at the Leavenworth and Winthrop hatcheries. The viscera fed to this group of fingerlings were taken at random from the cold-storage facilities at Leavenworth; no attempt was made to feed this group viscera from a designated lot. Only 2 of 12 troughs of fingerlings became infected in this group.

Airborne and aquatic organisms.--No positive evidence was obtained to incriminate either airborne or aquatic organisms as possible vectors of this disease. However, no comprehensive survey was made during the epizootic to determine if there was a correlation between the incidence of the disease and the aquatic and airborne populations. The water supplies other than those originating from springs or wells (table 2) were obtained from cold, fast-flowing mountain streams where the plankton population was comparatively low. It was demonstrated that in at least one instance no aquatic vertebrate was acting as a vector for the virus. At the Cook hatchery in 1953, the water supply was obtained from a spring which originated approximately 100 yards from the hatchery. No vertebrates lived in this water supply prior to its entrance into the hatchery.

Eggs.--It is possible that the disease is transmitted from the adult to the fingerling sockeye salmon through the eggs. Table 3 lists the rivers in which adult sockeye return to spawn and the hatcheries in which their progeny were reared in 1951-53.

Epizootics occurred at all five hatcheries although the eggs came from adults from four different rivers. Three of these four rivers are widely separated geographically. As shown in table 3, fingerlings became infected at one hatchery, but not at another, although the original source of eggs was the same for both hatcheries.

Environmental Factors

Water temperatures.--Seven of the eleven epizootics occurred when the water temperature was between 45° and 48° F., but four epizootics occurred when the temperatures were between 50° and 53° F. (table 1). Four of the epizootics occurred when the water temperatures were rising, five when the water temperatures were relatively constant, and two when the water temperatures were declining.

Time of year.--Of the eleven epizootics investigated, one started in March, three in April, two in May, three in June, one in July, and one in August (table 1).

Shock.--During June and July the fish were subjected to more handling and movement than earlier or later in the year. During this period the fingerlings were subjected to the shock of being moved into other troughs or ponds. In addition, during this period, and in some instances in early May, weekly prophylactic treatments with pyridylmercuric acetate were given to prevent gill disease. Possibly, the shock suffered by the fish from either the moving or the prophylactic treatments was sufficient to make them more susceptible to the virus disease.

Host Factors

Age.--Epizootics were most prevalent in 5- to 6-month-old sockeye fingerlings. Six of the eleven epizootics occurred in populations of this age, three in populations 3 to 4 months old, and two in populations

Table 2.--Hatchery water supplies

HATCHERY	1951	1952	1953
Leavenworth	Icicle Creek Wells	Icicle Creek	Icicle Creek
Winthrop	Methow River Spring	Methow River	Methow River
Entiat	--	--	Spring water
Issaquah	--	--	Issaquah River
Cook	--	Little White Salmon River	Spring water

Table 3.--Rivers from which eggs were obtained

HATCHERY	1951	1952	1953
Leavenworth	Little Wenatchee White	Little Wenatchee White	Little Wenatchee White
Entiat	*Little Wenatchee White	*Little Wenatchee White	*Little Wenatchee White
Winthrop	Little Wenatchee White Methow	Methow	Methow
Cook	*Little Wenatchee White	Little Wenachee White	Little Wenatchee White
Issaquah	*Issaquah	*Issaquah	Issaquah

* Fish from these eggs not diseased.

7 to 8 months old. No information is available concerning epizootics in fingerlings reared under natural conditions, or in sockeye salmon from the time they are released from the hatcheries until they return to spawn.

Size.--The onset of the disease occurred in populations in which the usual length of the fish was between 1 and 4 inches. Five of the epizootics occurred in fish 2 to 3 inches long, three in fish 1 to 2 inches long, and one in fish 3 to 4 inches long. At the Leavenworth hatchery in 1953, the fish were segregated into two size groups. In one group the fish were 1 to 2 inches long, and in the other group 2 to 3 inches long. When the mortality rates of infected populations, each with a different average size, were compared no significant differences could be found. However, in any infected population, in general, the majority of the survivors were the smaller, weaker fish.

Heredity.--This disease has not been limited to one race of sockeye, but has occurred in the progeny of adults which returned to spawn in four different rivers. Moreover, the disease has occurred in fish of this species which spend their entire life cycle in fresh water as well as in other fish of this species which spend only the first 12 to 24 months of their life cycle in fresh water and most of the next 2 years in salt water.

Although the disease was manifest in four different races, the mortality was significantly less in one race in 1952 and 1953. Although most of the fish from the native stock at Winthrop, reared at that hatchery, died in the epizootic of 1951, only 45 to 55 percent of the fish from the native stock died during the epizootics at Winthrop in 1952 and 1953.

EXPERIMENTAL WORK

Objectives

To support the epizootiological findings we initiated an experimental program, but with limited personnel and time we realized we had our choice of concentrating our efforts on one small facet of the problem or conducting a broad exploratory program, the results of which would guide in directing future research in the most profitable direction. We accepted the broad general approach for the present set of experiments, hoping it would reveal facts which would allow us to concentrate upon more specific facets in future investigations.

The objectives of our program were to determine the effects of the chemical, physical, and environmental factors on the disease, and also, to investigate the basic nature of the etiological agent and to determine the virulence and specificity of the agent and the immunities of blueback salmon and other species of fish.

The following sections are an account of the experimental work on this disease.

Methods and Materials

Inoculum

Source.--The original source of the infectious agent was from dead or moribund fish from naturally infected hatchery populations. In the majority of the experiments, the original source of the virus was from the 1952 epizootic at Cook, the 1953 epizootics at Issaquah and Leavenworth. The inoculum was prepared from moribund fish and those that died during an epizootic and from artificially infected fish inoculated with serial transfers of the infectious material. Samples of the livers and some of the eggs of 2,000 apparently healthy adults which returned to spawn in the Little Wenatchee and White Rivers in the fall of 1953 were tested.

Preparation.--In general, the inoculum from dead or moribund fish was prepared as follows: whole fish were blended in a Waring blender or an Osterizer for 30 to 60 seconds; the blended suspension was centrifuged for three to five minutes at about 2,000 r.p.m.; the supernatant was diluted usually 1 to 100 with sterile tap water and passed through a bacterial filter. The filtered suspension was tested for the presence of bacteria by streaking on a nutrient agar plate.

The bacterial filter used routinely in experiments was a 7-pound Mandler filter. Other filters used less frequently included vacuum Seitz, ultrafine sinter glass, No. 4 Pasteur-Chamberlin, and Millipore.

Frequently, healthy fish were inoculated with various dilutions of the infectious agent to determine the greatest dilution that would kill healthy fish. This procedure is referred to as titration. Hundredfold dilutions from 10^{-1} to 10^{-10} were made when infectious material was titrated. Sterile tap water was used as the diluent fluid.

To determine the organ specificity of the infectious agent, various organs were removed from infected fish, homogenized in a 50-ml. tissue blender, filtered through a Millipore filter, diluted from 10^{-4} to 10^{-14}, and each dilution inoculated into 25 fish.

An attempt was made to determine whether adult sockeye salmon which returned to spawn in the Little Wenatchee and White Rivers could be acting as carriers of the agent responsible for the epizootics at the sockeye hatcheries. Approximately 2,000 adults which returned to spawn in these two rivers were tested for this possibility by making 76 pools of their livers, blending and diluting each pool to 10^{-1}, and inoculating the unfiltered suspension into 25 healthy fingerlings. Fish which died after inoculation in ten groups of fingerlings were tested to determine if the

agent responsible for the deaths was filterable and serially transmissible. Dead fish from these groups were blended, diluted to 10^{-1}, filtered through a 7-pound Mandler filter, and inoculated into 10 additional groups of 25 sockeye fingerlings each. Infectious material from these inoculated fish was transmitted through three additional serial transfers.

An attempt was also made to determine whether the infectious agent could be transmitted from adults through apparently healthy eggs. A sample of 100 fertilized eggs was taken from 100 groups of 30,000 eggs, each obtained from about 12 females. Each sample was homogenized in a Waring blender, diluted 1 to 10 with sterile tap water, and injected into 25 healthy fingerlings.

Routes of inoculation.--The route of inoculation used depended upon the objectives of the experiment. Infection is possible by intraperitoneal or intramuscular inoculation, feeding infected tissue, contact, and by suspending healthy fish in a filtered or nonfiltered suspension of blended, diseased fish.

Intraperitoneal inoculation was used for most of the titering and many of the other experiments. The amount of the inoculum given depended upon the size of the fish. One- to three-month-old fingerlings received 0.05 ml., 2-to 12-month-old fish 0.10 ml., and adult fish 10 ml. of suspension. Concentrations used varied from 10^{-1} to 10^{-12}.

To transmit the virus by feeding, a mixture of 50 percent normal diet and 50 percent ground, infected fish was usually fed for a period of 3 days.

Fish were also infected by placing moribund or dead fish in contact with healthy fish, or by holding diseased fish in the water supply above the noninfected group.

Transmission by suspension was carried out by placing healthy fish in filtered or nonfiltered suspensions of infected fish for periods of 15 to 60 minutes. These suspensions ranged in dilution from 10^{-2} to 10^{-8} and were prepared in the same manner as the material for intraperitoneal inoculation.

In addition, experiments were conducted at two of the hatcheries to determine whether the disease could be spread from infected to healthy populations by the use of common cleaning and feeding equipment. The healthy fish were in troughs adjacent to the infected ones, and no attempt was made to rinse, clean, or sterilize the equipment used during the course of the experiment.

Stability of virus.--Diseased material was subjected to freezing, heating, suspension in glycerol, and to different hydrogenion concentrations to determine the effect upon the infectious agent.

The temperatures used in freezing were 0° and -40° F., which will be discussed in greater detail when pertinent.

The effect of elevated temperatures on a suspension of the infectious material was tested by heating aliquots of a suspension at 10° intervals from 30° to 100° C. for periods up to 15 minutes before inoculation. Whole diseased fish were also subjected to the same temperatures for 15 minutes and then placed in contact with healthy fish.

Aliquots of a virus suspension were adjusted from pH 4.0 to pH 10.0 by the addition of 0.1 N HCl or 0.1 N NaOH. Each aliquot was incubated at room temperature for 1 hour and then returned to neutrality by the addition of equivalents of 0.01 N HCl or NaOH. Before inoculation each aliquot was adjusted so that the final dilution was 10^{-1}.

Fish

Source.--The majority of the experimental fish were 6- to 10-month-old fingerling sockeye salmon which were reared at the Leavenworth hatchery. In addition, fish of the same age groups from hatcheries at the University of Washington, Entiat, Winthrop, and Cook were also used.

Adult salmon used in these experiments were trapped at the Rock Island Dam on the Columbia River and transported to the Leavenworth hatchery where they were held in rearing ponds for 2 months prior to spawning.

Other fingerling populations used in these experiments included rainbow trout and chinook and silver salmon. The rainbow trout were obtained from either the University of Washington or the Winthrop hatchery, and the silver salmon were reared at the University of Washington.

Diseased populations used in the studies in the summer of 1953 were from the Leavenworth hatchery.

Numbers.--There was a considerable variation in the number of fish used in any one experiment, ranging from a minimum of 25 fish to a maximum of 3,000 fish per experiment. In most of the experiments where infectious material was titrated, 25 fish were inoculated with each dilution. When the infectious material was not titrated, a minimum of 200 fish were used per experiment.

Environmental Conditions

Water temperature.--The water temperatures during the routine experiments varied from 48° to 62° F. For specific water-temperature experiments the water was held at 40°, 50°, 60°, and 68° F. with a 2-degree variation.

Chemotherapy.--Healthy 7-month-old sockeye fingerlings averaging 3 grams and having a length of 2 to 3 inches were fed a diet which had incorporated one of the following antibiotics. These fish were fed daily an amount of food equal to 8 percent of their body weight. The medicated foods were given for 6 consecutive days before inoculation with the virus and for 20 consecutive days after inoculation. The medication used was as follows:

Antibiotic	Dosage
Penicillin	1,000,000 units per 200 gm. of feed
	2,000,000 " " " " " "
Terramycin	250 mg. per 70 gm. of feed
	500 " " " " " "
Chloromycetin..................	1 gm. per 100 gm. of feed
	2 " " " " " "

Naturally infected fish were fed neoarsphenamine and atabrine at a level of 150 mg. per pound of food per day. Infected populations were also fed the following sulfonamides for 13 consecutive days at a level of 10 gm. per 100 lbs. of fish per day: sulfadiazine, sulfamerazine, and sulfamethazine.

Nutrition.--Seven- to nine-month-old healthy sockeye salmon fingerlings at the Leavenworth hatchery in 1953 were starved for a period of 2 months. At the end of 2 weeks, and again at the end of 2 months, the fish were tested for susceptibility to the virus.

Crowding.--The relation of host population densities to host susceptibility was investigated. Four troughs were divided into sections so that the section in the second trough had three times the volume of the first, the third, six times the volume, and the fourth, nine times the volume. Two hundred fish were placed in each trough and exposed to the disease by placing dead, infected fish at the head of each trough.

In another experiment, two groups of fish were moved from rearing ponds to holding ponds where both the area and the volume of water were estimated to be increased 20 times that of the rearing ponds. One group of 200,000 fingerlings at the time they were moved showed no symptoms of the disease. A second group of 400,000 fingerlings which was moved to the second holding pond had an excessive mortality rate and showed symptoms of the disease 1 week before they were moved. When these fish were moved,

the daily mortality rate just before moving was 1 percent a day. Five other groups of infected fish, each with a population of 200,000 were retained in five rearing ponds as controls.

Results

Characteristics of the virus

Demonstration of causative agent.--Numerous attempts were made to culture this agent on various bacteriological media; however, neither bacteria nor any other microorganisms were consistently demonstrated to be present in diseased fish. All attempts made to demonstrate the infectious agent in diseased tissues were negative -- either by examining wet mounts with the phase-contrast microscope or stained smears with the light microscope.

Infectious nature.--Although it was not possible to demonstrate the causative agent of this disease, either by direct observation or by cultural techniques, the infectious nature of the etiological agent was easily proved. Sockeye-salmon fingerlings were readily infected orally, intraperitoneally, and by contact. The relative effectiveness of each route of infection is shown in figure 6. In one experiment, one group was inoculated by intraperitoneal inoculation of a 10^{-2} suspension. In another experiment, groups of healthy fish were infected by one of the following methods: Intraperitoneal inoculation of a 10^{-2} suspension, holding the healthy fingerlings in a trough with sick fingerlings, feeding the fish a 1:1 mixture of ground, infected fish, or by holding dead fish in the water supply of a healthy group of fingerlings. Although the intraperitoneal inoculation was the most effective method of infection, the fish were easily infected by oral inoculation and by two methods of external inoculation. It was likewise demonstrated that this infectious agent was serially transmissible. Serial transfers were made of the infectious material by inoculating a group of healthy fingerlings with a 10^{-2} suspension of the infectious material; when the fish showed symptoms of the disease they were sacrificed and a filtrate was prepared and inoculated into an additional group of healthy fingerlings. After 12 such serial transfers, with a final dilution factor of 10^{-30}, no decrease in virulence of the infectious agent was found.

Filterability.--In addition to showing that the agent was infectious, the experiments also demonstrated that the agent was filterable. The filtrate of a 10^{-2} suspension of diseased tissue retained its infectious nature after it had passed through any of the common bacterial filters.

Effect of storage.--For the laboratory study of any virus, it is desirable to have a homogeneous standard stock suspension of known virulence. For most virus studies, stock suspensions are stored in glycerine or frozen

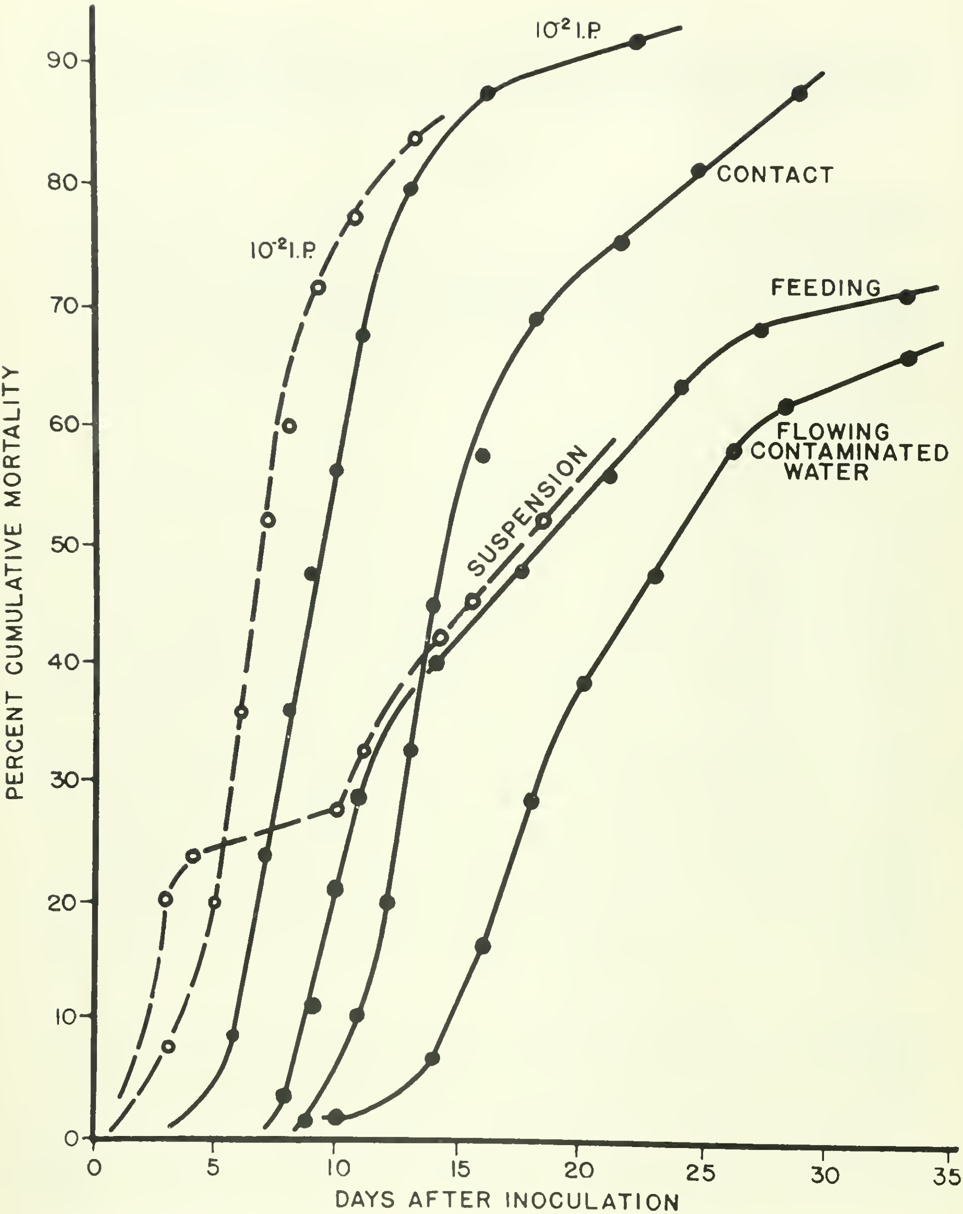

Fig. 6.--Variation in mortality patterns correlated with route of inoculation. Each curve represents a group of 200 fish infected by the indicated route.

at a low temperature. The viability of these suspensions frequently varies depending upon the temperature at which they are stored, the diluting media, the degree of desiocation before storage, and the period of storage.

An exploratory attempt was made in this investigation to obtain a frozen stock suspension of this virus. Initially, whole moribund fish were frozen and later thawed, blended, and filtered to obtain a suspension of the virus when needed for experimental purposes. This method was unsatisfactory since it was not possible to obtain a consistent, homogeneous suspension. Moreover, the titer of material obtained in this manner dropped from 10^{-6} before freezing to 10^{-2} after freezing, but maintained this degree of viability when stored at 0° F. for a period of 1 year. However, if the fish were thawed and refrozen more than once they became innocuous when inoculated into experimental fish.

In order that a more homogeneous and uniform suspension should be available for experimental use, moribund fish were blended, diluted 1 to 10 with tap water, centrifuged, and stored in small glass vials which were sealed with a gas-oxygen flame. These suspensions were then stored at 0° F. The infectious level of these suspensions dropped from 10^{-6} before freezing to 10^{-2} after freezing; the virulence of this suspension gradually decreased until 4 months after its preparation it was no longer infectious. It was recognized that the maintenance of a suspension of known virulence might be incumbent upon the temperature at which it was stored and the degree of protein protection if diluted. Therefore, a suspension from moribund fish was prepared by blending infected fish: one-half of the emulsion was diluted 1:3 and the other half diluted 1:10 before storage at -40° F. This suspension had a titer of 10^{-8} before freezing. Three hours after freezing the material diluted 1:10 had a titer of 10^{-1} while the material diluted 1:3 had a titer of 10^{-6}. The virulence of the latter material decreased rapidly until at the end of 2 months it had a titer of 10^{-2}. This infectious level decreased to 10^{-1} after the suspension had been held at -40° F. for a period of 1 year. Virus material, when stored in a 1:10 glycerol suspension at 12° C., remained viable for only 3 weeks.

Effect of heat.--The infectious agent was consistently killed if heated for 15 minutes at 60° C. However, in some experiments the infectious nature of diseased tissue was destroyed if heated for 15 minutes at 50° C. or for 3 minutes at 60° C.

Effect of pH.--Virus suspensions adjusted from pH 4 to pH 10 and incubated for 1 hour before being adjusted again to neutrality and then inoculated intraperitoneally into sockeye fingerlings were all virulent. The only noticeable difference in mortalities was found in the group inoculated with the suspension incubated at pH 4; here, only one-half the number of mortalities developed as compared with the other suspensions.

Host-Parasite Relation

Incubation period.--Experimentally, the incubation period of this disease varied with the amount of infectious material inoculated, virulence of the virus (titer), source of inoculum, route of inoculation, and the water temperatures at which the inoculated fish were held.

The maximum incubation period was not demonstrated in this investigation since it was impossible to determine whether the fish which died in the latter part of an experiment died from the original inoculum, or from a reinfection contracted by contact with fish which had become diseased during the initial phases of the experiment. However, deaths have occurred in groups of sockeye fingerlings up to 45 days after the original inoculation. The minimum incubation period was found to be 1 day.

In general, the higher the titer of the infectious material, the shorter the incubation period, and conversely, a corresponding increase in the incubation period as the dilution increased. When the titer of the infectious material was 10^{-6} at the start of the epizootic at Leavenworth in 1953, the incubation period was 3 to 5 days, but when the titer rose during the middle of the epizootic to 10^{-10}, there was a drop in the minimum incubation period to 1 to 2 days. Likewise, in the latter part of the epizootic at this hatchery, the titer dropped to 10^{-2} and the minimum incubation period increased to 7 to 10 days. When hundredfold dilutions of diseased tissue were made and inoculated into healthy fingerlings, the minimum incubation period in the fish inoculated with the 10^{-2} suspension was from 1 to 4 days shorter than in those inoculated with the highest dilution which was still infectious.

The incubation period was also contingent upon the route of inoculation. The difference in the incubation period with different routes of inoculation is seen in the comparison of groups of sockeye fingerlings, each infected by a different route of inoculation (Figure 6). The disease in those fish infected with material having a titer of 10^{-10} by intraperitoneal inoculation, or by being held in a suspension of infected tissue, had a shorter incubation period than when the fish were fed infected food or held in contact with dead fish.

In other experiments, using material having a lower titer, similar differences in incubation periods were found; however, with a decrease in titer there was an increase in the incubation period.

Change in virulence during an epizootic.--Two phenomena were found during the epizootic at the Leavenworth hatchery in 1953 indicating a change in virulence of the virus during the course of an epizootic. First, it was found that the virulence of the infectious agent increased shortly after the onset of an epizootic in any given trough, but decreased at the end of the epizootic. Second, it was found that fewer fish died in

troughs infected in the latter part of the epizootic than in troughs infected at the first part of the epizootic.

Within 2 weeks after the onset of the disease at the Leavenworth hatchery in 1953, and thereafter for the next 3 months, infected fish were taken at random from infected troughs and titrated into healthy sockeye fingerlings. Fifteen days after the onset of the disease on June 30, the titer of the infectious material was 10^{-6}; by July 3, however, the titer had risen to 10^{-10}. A titer of 10^{-10} was found through July, but by August 1, when daily mortalities started to decline, the titer had decreased to 10^{-6} and by August 13, it was 10^{-4}. In another week, the titer decreased to 10^{-2}. By October 1, the daily mortality rate had returned to nearly normal and no filterable infectious agent could be demonstrated in fish dying in the ponds or troughs (table 4).

It is believe that this change in titer of the infectious material was indicative of a change in the virulence of the causative agent rather than a change in the resistance of the fish to the disease. During the period when the titer was 10^{-2} the virus was serially transmitted twice and the titer returned to 10^{-6}.

Table 4.-- Variation of titer during course of the epizootic at Leavenworth hatchery in 1953

June 30	10^{-6}
July 2	10^{-8}
July 3	10^{-10}
July 16	10^{-10}
July 21	10^{-10}
July 23	10^{-10}
August 1	10^{-6}
August 13	10^{-4}
August 21	10^{-2}
September 26	infectious agent could not be demonstrated

In late August, a healthy trough became infected by contact from an adjacent trough. In an attempt to gain additional information on this apparent change in virulence, material from infected fish was titered periodically during the course of the disease. The periods at which the titrations were done is indicated by the arrows on figure 7. In contrast to the previous experiments, the titer did not rise, but remained at 10^{-6} through the first 22 days of the epizootic. The titer then dropped and remained at 10^{-4} through the 36th day of the epizootic. The titer on the 42nd day was 10^{-2} (each titration based on that of at least 50 dead or moribund fish).

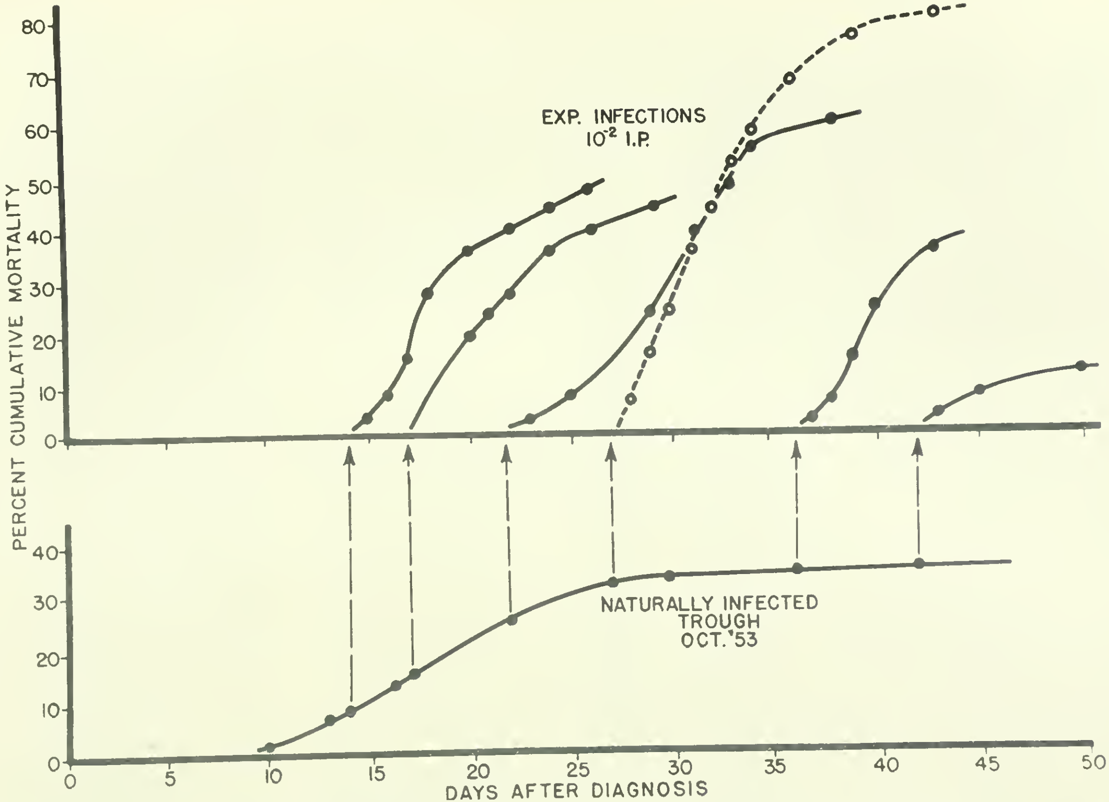

Fig. 7.--Variation of virulence of the virus during the course of the disease in one trough at Leavenworth hatchery in 1953. Lower curve represents mortalities in a trough population of 3,000 fish. Upper curves represent groups of 25 fish each inoculated with a 10^{-2} emulsion made from fish naturally infected as represented in lower trough. Dates at which infectious material obtained from naturally infected trough are indicated.

Further indications of a change in the virulence was found when the rates and cumulative mortalities of the groups of fish inoculated with a 10^{-2} suspension were compared (figure 7). The complete or final data show that 48 percent of the fish died when inoculated with material obtained on the 14th day, 56 percent on the 17th day, 84 percent on the 22nd day, 80 percent on the 27th day, 40 percent on the 36th day, and 12 percent on the 42nd day.

Species specificity.--All attempts to infect fish other than the 1- to 14-month-old sockeye salmon were negative. Chinook and silver salmon fingerlings were inoculated with filtered material intraperitoneally and by suspensions and were fed infected food. Rainbow fingerlings and adult suckers were given intraperitoneal inoculation only. It was not possible to recover the infectious agent from these fish.

Age specificity.--Experimentally, it was possible to infect 1- to 14-month-old sockeye salmon. No information is available between this age and the time they return to spawn.

Only cursory information is available concerning experimental infections in adult salmon. Forty adults were obtained from Rock Island Dam and held at the Leavenworth hatchery. Half of these were inoculated with a virus suspension and the other half held as controls. All 20 of the controls and 16 of the inoculated group died during the next 60 days, showing the presence of a fungus infection. Suspensions of the livers from these dead fish from both the inoculated and the control groups failed to produce any symptoms of the disease when inoculated into healthy fingerlings.

Although it was not possible to infect adults experimentally with a suspension of the virus, it was possible to recover a filterable infectious agent from adult sockeye salmon which had returned to spawn in the Little Wenatchee and White Rivers. Groups of healthy fingerlings were inoculated with 76 pools of livers from a total of 2,000 adult salmon. In the groups of fingerlings, 29 percent showed no mortality or less than 9 percent mortality, 59 percent of the groups had a 10- to 50-percent mortality, and 12 percent of the groups had mortalities in excess of 50 percent. Since the original inoculum was not filtered many of these deaths may have been due to bacteria. However, 9 of the 10 groups inoculated with the first serial transfer of filtered material from groups infected with the liver suspension showed a 40- to 98-percent mortality within 30 days after inoculation. No fish were lost in one inoculated group and none was lost in the noninoculated control group.

After three serial transfers, this filterable agent, when inoculated into a group of healthy fingerlings, caused a 56-percent mortality within 30 days after inoculation. The titer of this infectious material was 10^{-6}. No significant mortalities occurred in 100 groups of fingerlings which were inoculated with eggs spawned from these adult sockeye salmon.

Size specificity.--Fish of the same age group, but of different size groups were infected with the virus. No significant difference could be found in the mortality rate, incubation period, or symptoms.

Organ specificity.--When various organs from moribund fish which were infected during an epizootic at the Leavenworth hatchery in 1953 were titrated into healthy fingerlings, the highest concentration of the virus was found in the kidney and liver. The infectious agent also was demonstrated in the brain, spleen and gills, but the titer in these organs was significantly less than in the liver and kidney (table 5). The intestinal tract and muscle were devoid of any demonstrable infectious agent.

Table 5.--Titration of organs from infected fish in healthy sockeye fingerlings

Organ	Percent kill at each titration 19 days after inoculation		
	10^{-4}	10^{-6}	10^{-8}
Liver	100	56	0
Spleen	43.5	4.3	0
Kidney	59	75	0
Stomach, intestines	0	0	0
Brain	80	25	0
Gills	8.7	0	0
Whole Fish	42	0	0

Fish inoculated with tap water...control: 0.04 percent loss.
Noninoculated control: No loss.

In this experiment only losses in excess of 10 percent were considered significant.

Effect of water temperatures.--Groups of fish experimentally infected by the contact-suspension route of inoculation were held in constant temperatures of 40°, 50°, 60°, and 68° F. In this and other similar experiments 50° to 60° F. was shown to be the optimum temperature, but the virus remained virulent in fish held in 40° and 68° F. Fish inoculated at the three lower temperatures had mortalities in excess of 90 percent, but the fish inoculated at 68° had less than a 50-percent mortality (fig. 8).

Nutritional effect.--Fish which were starved for a 2-month period were not susceptible to the disease, although fish which were starved for only 2 weeks were susceptible.

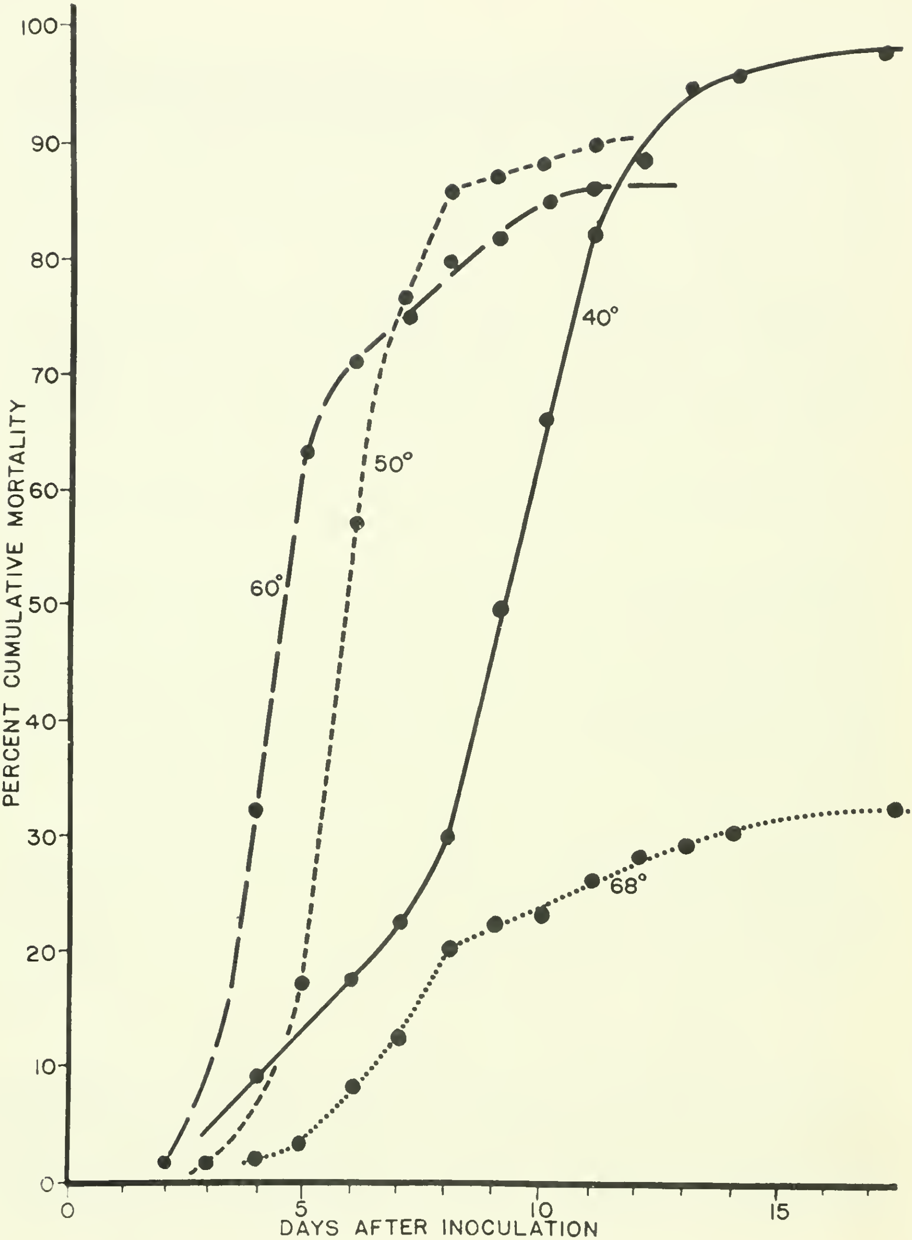

Fig. 8.--Effect of temperature on mortality rates in fish experimentally infected with the virus disease. Each curve represents the mortalities in groups of 200 fish. Fish were infected by the suspension method and held at the indicated temperatures until they died from the disease.

Immunity in recovered fish.--Fish which had recovered from the disease could be reinfected if inoculated with a high concentration of the infectious material. Previously infected fingerlings when inoculated with a 10^{-6} dilution of diseased tissues were not killed, but fish that had no previous history of the disease died when inoculated with this same material. When recovered fish were inoculated with a 10^{-1} suspension of this same material, 50 percent of them died. This apparent immunity may have been due to a natural immunity rather than to an immunity acquired from previous contact with the disease.

Effect of chemotherapy.--The oral administration of penicillin, terramycin, or chloromycetin to healthy fish before inoculation with the virus or after inoculation was not effective in controlling the virus disease. There was a slight suggestion that the fish treated with these antibiotics were more susceptible to the virus disease than the control fish which had not been treated with these antibiotics.

The mortality in naturally infected troughs treated with neoarsphenamine or atabrine was not altered; the symptoms of the disease were not alleviated, nor was the disease controlled by such treatments.

Effect of crowding.--Trough population densities did not change the susceptibility of the host to the disease. No significant difference was found in the mortality rates of groups of fish infected by contact and held in different volumes of water. Because of high predation and escapement in fish held in the holding ponds, it was not possible to keep accurate records to determine if the increased volume of water could help in reducing the mortality rates in these populations. However, approximately 40,000 fish were recovered from the 200,000 healthy fish originally placed in the first holding pond. This group of fish appeared healthy when moved, but became infected soon after being moved. Only 20,000 fish were recovered from the 400,000 fingerlings placed in the second holding pond.

Spread of the disease in the hatchery.--All attempts to spread the disease from infected to healthy troughs by use of contaminated feeding and cleaning equipment were negative.

DISCUSSION AND CONCLUSION

Etiology

The etiological agent in this disease was originally described by Rucker et al., (1953) as of possible virus origin. The present investigators were unable to demonstrate an infectious agent by direct microscopic examination of diseased tissue and fluids, nor could it be cultured on any of a number of different media. The agent has been shown to be serially transmissible, thus eliminating the possibility that it might be a nonliving,

toxic agent and it was demonstrated that it could be passed through bacteria-retaining filters. Thus, the present investigators have substantiated previous investigations, and it can be assumed that the agent is a virus. It is proposed that this agent be referred to as the sockeye virus.

Source of the Virus

Although it cannot be stated with certainty that the virus was introduced to fingerling populations through fish products, enough evidence has been obtained to warrant questioning the advisability of feeding untreated fish products to fingerling sockeye salmon. Theoretically, it is reasonable to believe that adult fish will have acquired sufficient immunity to many diseases thereby making them resistant in some degree to these diseases. The degree of resistance to a disease will certainly vary, depending upon both the acquired and natural immunity of the fish. Therefore, depending upon this degree of immunity, the adult sockeye may be completely resistant to some diseases, or resistant enough that they will show no clinical symptoms of the disease after being exposed to it. In the latter instance, the fish, although showing no clinical symptoms, could act as carriers of the disease. If salmon fingerling populations are fed fish products from adult salmon which are infected and in this manner act as carriers of the disease, it may be expected that the fingerling populations will become infected. Additional credence was given this possibility when it was demonstrated that a filterable infectious agent could be recovered from apparently healthy adults which returned to spawn in the Little Wenatchee and the White Rivers in the State of Washington. It is entirely possible that these adults were infected with the same infectious agent as caused the high mortalities in sockeye fingerling populations.

Since it is known that sockeye fingerling populations became infected when fed infected food, the use of fish products made from adult salmon, possibly diseased, may provide the original entrance of the etiological agent into the hatcheries. Unfortunately, immunological techniques have not been developed for positive identification of the sockeye virus; therefore, it is impossible to state unequivocally that the filterable agent recovered from the adults was identical to that recovered from the fingerling populations during an epizootic. However, the investigation showed that this possibility does exist.

Additional evidence to suggest that the sockeye virus might have been introduced into hatchery populations in the food was found at the Entiat hatchery in 1953. As previously described, at this hatchery two groups of fingerlings were fed two separate lots of salmon viscera from the same source. The only two troughs of fish in the first group which did not become infected were those fed the straight meat diet in which no fish products were incorporated. To further substantiate this theory was

the fact that the two groups of fish became diseased at different times; the second group had fewer infected troughs than did the first group of fingerlings. Possibly, this differential was due to the fact that each group was being fed viscera from different lots. Also, it might be that diseased viscera was fed in May to the first group, while the second group was not fed infected viscera until late in June or early July. Moreover, since the fish in more of the troughs in the first group became infected than in the second group it is entirely possible that more diseased viscera was fed to the first group than to the second.

Since it was impossible in this investigation to recover the virus directly from the viscera, and because all troughs of sockeye fingerlings in any one hatchery did not become infected, it may be assumed that all batches of the viscera were not contaminated with the virus. Moreover, it may be assumed that the food was not completely homogenized before feeding and that the infectious agent was irregularly distributed in the food.

No evidence has been obtained to suggest that the virus is transmitted from the adults to the fingerling salmon through the egg. Records were not available to allow the following of eggs from one female or several females to determine whether there was any indication that the virus was transmitted in this manner.

Likewise, no strong evidence has been obtained to indicate that the virus was introduced into a hatchery population through aquatic or airborne organisms. It is believed, however, that aquatic vertebrates do not act as vectors in this disease. This conclusion is based on the evidence that no vertebrates were present in the water source at one hatchery during the epizootic and the failure to demonstrate experimentally that species of fish other than O. nerka are susceptible or can act as vectors.

Although it is quite possible that an aquatic vertebrate was not acting as a vector in this disease, it is conceivable that either aquatic or airborne insects may have been serving as vectors. However, since the rivers from which the waters were obtained were widely separated geographically, it seems highly questionable whether a common insect would be found in all of these water supplies.

It would be expected that if an aquatic or airborne population were acting as a vector in this disease, and if all troughs in a hatchery did not become infected, the infected troughs would be distributed at random throughout the hatchery. This was not the case.

Why the majority of the epizootics started during the period from May through July is not known. If the virus was introduced into the hatchery populations through the eggs or the food, it would be expected that the incidence of disease during these months would be no higher than

in other months, unless there were factors during this period which predisposed the population to the disease. Experimentally, it has not been possible to demonstrate the existence of such factors. If the disease were introduced by aquatic or airborne vectors, the higher incidence during these months could possibly be correlated with an increase in insects possibly acting as vectors during this period. It is interesting to note that when the percentage of the troughs infected at the Leavenworth station in 1953 is plotted against time, a sigmoid curve is obtained (fig. 9), which suggests a limiting factor during the course of the epizootic. Possibly this limiting factor could be the increase and decrease of an insect population which serves as a vector in the disease.

It is not clear why only 69 of the 132 troughs at Leavenworth in 1953 became infected and why all but two of these troughs became infected between June 15 and July 15. All viscera were removed from the diet on June 22, but fed again to one-half of the remaining healthy fish starting July 30. None of these troughs became infected though they were fed a diet containing 50 percent viscera. It would seem likely that if the virus had been introduced into the hatchery population through the viscera, some of the viscera fed after this date would have been infective. It is possible that by the end of July the resistance of the fish had increased enough to make them immune to the amount of virus carried in the frozen viscera, or it is possible that none of the viscera fed at this time contained virus. This removal of viscera from the diet may be the limiting factor of the sigmoid curve (fig. 9). If some insect were acting as a vector this phenomenon might be explained by the increase of the population during the period when the majority of the troughs became infected and the subsequent decrease in the insect population during the period when only 2 of the 52 remaining healthy troughs became infected.

Of possible significance is the climatic condition at the hatcheries and the time of year during which the epizootics have occurred. Two of the hatcheries are located in western Washington where the climate is milder and wetter than in eastern Washington where three of the hatcheries are located (fig. 1). The onset of the epizootics in the three eastern Washington hatcheries was almost simultaneous (see table 1). The epizootic in the western Washington hatchery in 1952 occurred 2 to 3 months after the onset of the epizootics in the eastern Washington hatcheries. In 1953, the epizootics in western Washington hatcheries occurred approximately 30 to 40 days before the onset of epizootics in eastern Washington hatcheries.

Control of the Disease

Because viruses are intracellular parasites dependent upon the metabolism of the host cell for their nutrition, most virus diseases cannot be controlled, nor can the symptoms be alleviated by chemotherapy. Because of this and because of the work done in the present investigation where it was not possible to control the disease by chemotherapy, it is

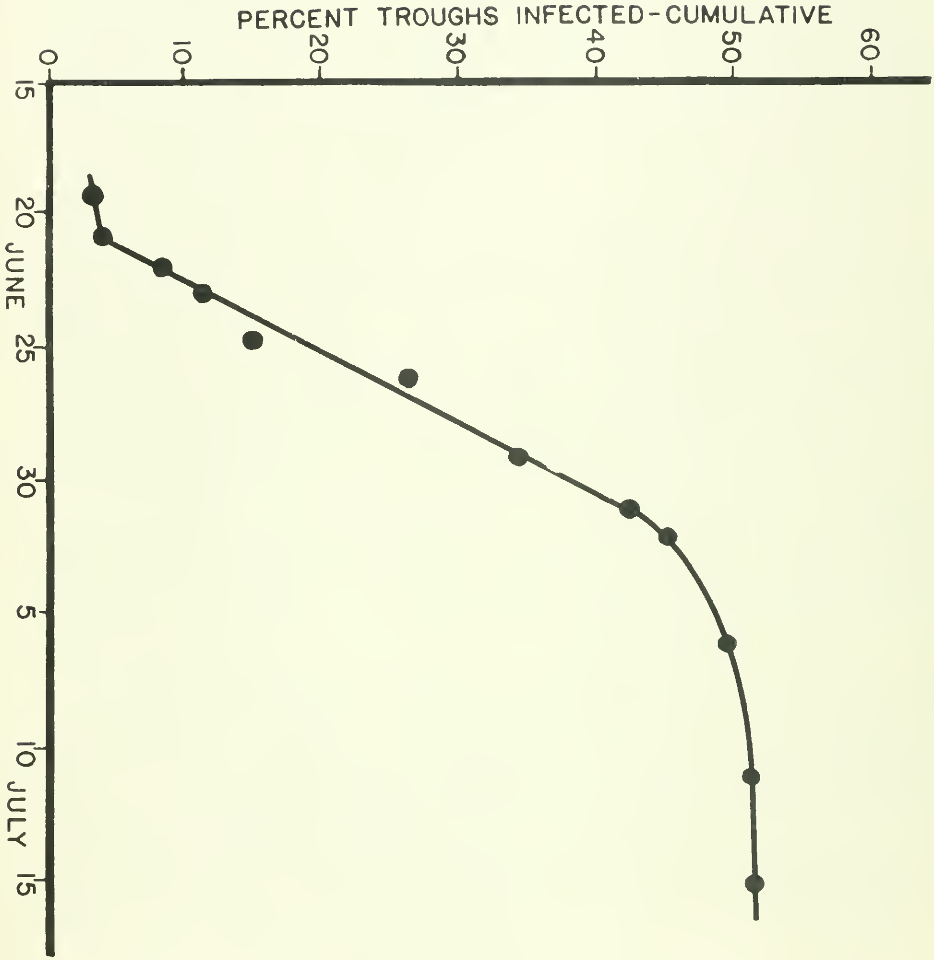

Fig. 9.--Epidemic at Leavenworth hatchery - 1953. Curve represents the total number of troughs in which there were infected fish at the indicated dates.

felt that an extensive investigation of this subject offers little promise for the control of the disease.

This investigation showed fairly conclusively that the disease spread rapidly through a population in a trough or a pond, once one or more fish became infected. Once a trough or pond became infected, reducing the number of fish per volume of water, chemotherapy, or any other change in their environmental conditions did not alter the course of the disease significantly.

Although Rucker et al., (1953) indicated that the disease was easily spread from diseased to healthy troughs, neither experimental results nor observations during the majority of epizootics substantiated those findings. Except for the original epizootic (Leavenworth) described by Rucker et al., and the epizootic at Cook in 1953, it did not appear that the disease was disseminated from trough to trough by fish-cultural procedures as carried on by hatchery personnel. However, at these two hatcheries when the disease occurred, the fish were so small they could have been transferred unnoticed into the healthy troughs in the bristles of the cleaning brush. It is believed by the present investigators that once the fish become so large that this danger no longer exists, the dissemination of the disease by contaminated cleaning and feeding equipment is slight. However, it must be emphasized that hatchery personnel should still take every sanitary precaution to prevent the spread of the disease.

The most promising method for control of the disease, at the present time, is the elimination of the source. Should the source be found to be aquatic or airborne vectors, or should it be demonstrated that the virus is being transmitted from the adults through the eggs to the fingerlings, the determination of a control measure will, no doubt, be a laborious and lengthy procedure. Should it be demonstrated that the virus is being introduced by the feeding of fish products, the elimination of the disease in hatchery-reared fish should be simple.

ACKNOWLEDGMENTS

The authors thank all those to whom they are indebted for having made this study possible. Part of this work was done at the Fisheries Center at the University of Washington, while the remaining experiments were conducted at the U. S. Fish and Wildlife hatcheries at Cook, Entiat, Leavenworth, and Winthrop, all located in the State of Washington. We thank Dr. Van Cleve, Director of the School of Fisheries; and Mssrs. Hagen, Garlick, Gastineau, Burrows, and Bernier of the U. S. Fish and Wildlife Service for making these facilities available. Our thanks are also due Mssrs. Johnson and Palmer who helped plan and execute some of the experiments, and Mssrs. Soderstrom and Holliday who very kindly gave invaluable technical assistance for which we are particularly grateful.

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